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Research paper

Immunoglobulin gene expression in umbilical cord blood-derived CD34$^+$ hematopoietic stem/progenitor cells☆

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ABSTRACT

Recently, immunoglobulin (Ig) expression was reported in a variety of non-B lineage cells, including myeloid cells. We assessed whether hematopoietic stem/progenitor cells (HSC/HPCs) can express Ig. With Gene Expression Omnibus (GEO) microarray database analysis, we found that IGHM was expressed with the highest frequency and level in umbilical cord blood CD34$^+$ HSC/HPCs, followed by IGK@, IGHE, IGHD, IGHG1, and IGHA1, while IGL@ was nearly not expressed. Ig expression was further confirmed by molecular experiments and immunofluorescence. Moreover, HSC/HPCs-derived Ig displayed restricted/biased usages and V_HDJ_H rearrangement patterns. These results suggest that Igs, especially IgM, may have a role in CD34$^+$ HSC/HPCs function.

1. Introduction

Immunoglobulin (Ig) is widely accepted as a product of B lineage cells and plays an important role in adaptive immunity to recognize and neutralize specific antigens such as bacteria and viruses. It is generally present as a tetrameric structure with two-fold symmetry, consisting of two identical heavy (H) chains and two identical light (L) chains connected by disulfide bridges (Woof & Burton, 2004). There are five classes of H chains, α, δ, ε, γ, μ, and two types of L chains, kappa (κ) and lambda (λ), in human Igs. Each Ig chain has a variable (V) region with extensive diversity and a constant (C) region that is generally conservative. According to the type of H chains, Igs are classified as five subclasses, IgA (α), IgD (δ), IgE (ε), IgG (γ) and IgM (μ) (Janeway et al., 2001).

Human Ig genes are located in three separate loci, known as IGK@, IGL@, and IGH@, on chromosomes 2 (Malcolm et al., 1982), 22 (Erikson et al., 1981; McBride et al., 1982a), and 14 (Kirsch et al., 1982; McBride et al., 1982b), respectively. Each of these loci contains V and C genes. In the light chain loci IGK@ or IGL@, the V genes contain two separate segments, V_κ and J_κ (or V_λ and J_λ) (Blackwell et al., 1986). In the heavy chain loci, there are three separate segments, V_H, D, and J_H (Early et al., 1980). Multiple copies of these V, (D) and J gene segments contribute to the great combinational diversity of Igs.

V(D)J recombination has been believed to occur only in B lymphocytes and plasma cells. However, during the last decade, many researchers have reported Ig expression in a variety of non-lymphoid cells, including epithelial cancer cells (Qiu et al., 2003; Babbage et al., 2006; Chen & Gu, 2007; Zheng et al., 2007; Zheng et al., 2009), normal epithelial cells (Jiang et al., 2015), central neurons (Huang et al., 2008; Niu et al., 2011), germ cells of mouse testis and epididymis (Huang et al., 2009), and lactating mouse mammary epithelial cells (Zhang et al., 2010). Furthermore, growing evidence has revealed that non-B derived Ig is involved in cancer cell survival and tumor development (Qiu et al., 2003; Babbage et al., 2006; Chen et al., 2010). More recently, we have reported that γ chain (Qiu et al., 2013), μ chain (Huang et al., 2014) and Igκ (unpublished data) genes were expressed in acute myeloid leukemia (AML) cells. Moreover, the μ chain, but not the γ chain, was also found to be expressed in mature myeloid cells (monocytes and neutrophils) from patients with non-hematopoietic neoplasms and healthy individuals. These results indicate that Igs can be expressed by both immature myeloblasts and mature myeloid cells. However, it is unclear if Igs can be expressed by hematopoietic stem/progenitor cells (HSC/HPCs).

Abbreviations: AML, acute myeloid leukemia; C region, constant region; HSC/HPCs, hematopoietic stem/progenitor cells; IF, immunofluorescence; Ig, immunoglobulin; RBE curve, rank-based gene expression curve; UCB, umbilical cord blood; V region, variable region.

☆ Authorship contributions: XQ conceived and supervised the study; JL provided the samples; JL, MX, CW, ZG, and CZ designed and performed experiments; PW downloaded and screened the GEO data sets; MX analyzed data and wrote the manuscript; CCY and XQ made manuscript revisions.

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Table 1
PCR primers.

Gene		Primers	Product size (bp)
IGHM		Forward 5′-CCCGACTCCATCACTTTCTC-3′ Reverse 5′-TGGTCTGCTTCAGTGGCG-3′	850
IGHD		Forward 5′-CACCTGACCTGGGAGGTGGCTGGG-3′ Reverse 5′-CAGGAGCCACGAGGCCGCCTCGGG-3′	279
IGHG1	First round	Forward 5′-AGGACTCTACTCCCTCAGCAG-3′ Reverse 5′-TCAGGCTGACCTGGTTCTTG-3′	566
	Second round	Forward same as first round Reverse 5′-ACACGGTACGTGCTGTTGTA-3′	375
IGHE	First round	Forward 5′-GGACGTGGACTTGTCCACCGCCTC-3′ Reverse 5′-CCGGGGCAGCACGGCGGGCCGCTGG-3′	506
	Second round	Forward 5′-GCACTGGCTGTCAGACCGCACCTAC-3′ Reverse 5′-GGTGGAGTGGTTCACAGGCTTCCC-3′	253
IGHA1	First round	Forward 5′-TGTGACCTGGAGCGAAAGC-3′ Reverse 5'-CGGGATGCCCAAGTCAGGTA-3′	732
	Second round	Forward same as first round Reverse 5′-AACAGCGCTCTTCCCACTTG-3′	400
IGK		Forward 5′-GACATCGAGCTCACCCAGTCTCC-3′ 5′-GAAATTGAGCTCACGCAGTCTCCA-3′ Reverse 5′-CGGGAAGATGAAGACAGATGGTGC-3′	About 357
CD19		Forward 5′-AAGGGGCCTAAGTCATTGCT-3′ Reverse 5′-CACGTTCCCGTACTGGTTCT-3′	379
CD34		Forward 5′-TGAAGCCTAGCCTGTCACCT-3′ Reverse 5′-CGCACAGCTGGAGGTCTTAT-3′	200
gDNA-IGHV	First round	Forward 5′-ACACGGCYSTGTATTACTGT-3′ Reverse 5′-TGAGGAGACGGTGACC-3′	About 60–110
	Second round	Forward same as first round Reverse 5′-GTGACCAGGGTNCCTTGGCCCCAAG-3′	

In this study, we assessed Ig expression in umbilical cord blood (UCB) $CD34^+$ HSC/HPCs (Cutler & Ballen, 2012) using Gene Expression Omnibus (GEO) microarray database analysis. Ig expression was further confirmed by molecular experiments and immunofluorescence (IF). We found for the first time that Ig genes, especially IGHM, the earliest Ig class that is expressed during evolution, were expressed in UCB $CD34^+$ HSC/HPCs. Moreover, HSC/HPCs-derived Igs displayed restricted/biased V_HDJ_H rearrangement patterns. These results suggest a role of Ig expression in HSC/HPCs function.

2. Materials and methods

2.1. Data set collection

Microarray data set files on Affymetrix Human Genome U133 plus 2.0 platform (GPL570) were downloaded from the GEO database (http://www.ncbi.nlm.nih.gov/geo/) using GEOquery package in the R statistical programming environment (Davis & Meltzer, 2007). Based on annotations, microarrays related to UCB $CD34^+$ HSC/HPCs were screened by text mining and confirmed manually. A global quality control (QC) analysis of raw data quality was performed using the BioConductor package "simpleaffy" (Wilson & Miller, 2005). Data sets containing extreme values from at least one QC stat were excluded.

2.2. Isolation of UCB $CD34^+$ HSC/HPCs

UCB samples of ten healthy individuals were provided by the Department of Obstetrics at Beijing Jishuitan Hospital, China. Mononuclear cells were isolated by density gradient centrifugation using lymphocyte separation medium (1.077 g/L, Huajing Biotechnology Co., Ltd., Shanghai, China). Erythrocytes were lysed by incubation with 2–3 mL red blood cell lysis buffer at 37 °C for 10 min. $CD34^+$ HSC/HPCs were purified by magnetic cell sorting using the Diamond CD34 Isolation Kit (Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer's instructions.

Table 2
PCR conditions.

Gene			Denaturation	Annealing	Elongation	Cycles	
IGH-C region CD19 CD34		94 °C 5 min	94 °C 30 s	55 °C 30 s	72 °C 45 s	38	72 °C 7 min
IgKV		94 °C 5 min	94 °C 30 s	60 °C 30 s	72 °C 30 s	3	
			94 °C 30 s	58 °C 30 s	72 °C 30 s	3	
			94 °C 30 s	56 °C 30 s	72 °C 30 s	3	
			94 °C 30 s	54 °C 30 s	72 °C 30 s	3	
			94 °C 30 s	52 °C 30 s	72 °C 30 s	3	
			94 °C 30 s	50 °C 30 s	72 °C 30 s	3	
			94 °C 30 s	48 °C 30 s	72 °C 30 s	20	72 °C 7 min
gDNA-IGHV	First run	94 °C 5 min	94 °C 30 s	55 °C 30 s	72 °C 30 s	30	72 °C 7 min
	Second run	94 °C 5 min	94 °C 30 s	57 °C 30 s	72 °C 30 s	35	72 °C 7 min